WEST Search History

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	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR		
☐	L16	L4 and ((Allen or Farrer).in.)	3
☐	L15	L14 same (host adj cell)	19
☐	L14	L12 same vector	80
☐	L13	L12 and vector	95
☐	L12	L4 same operatively	97
☐	L11	6506559.pn.	2
☐	L10	L8 and (graham.in.)	0
☐	L9	=2002	7
☐	L8	L7 and pharmaceutical	409
☐	L7	L6 and (host adj cell)	543
☐	L6	L4 and vector	750
☐	L5	L3 and promoter	87
☐	L4	(siRNA or RNAi or siNA) same promoter	800
	DB=USPT; PLUR=YES; OP=OR		
☐	L3	L2 and (host adj cell)	97
☐	L2	L1 and vector	441
☐	L1	graham.in.	9963

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Set     Items   Description
S1      29295   (RNAI OR SIRNA OR SINA OR DSRNA)
S2       1274   S1 (S) PROMOTER
S3        440   S2 (S) VECTOR
S4         76   S3 (S) (HOST (W) CELL)
S5          5   S4 NOT PY>2002
S6          5   RD (unique items)
S7          0   S2 AND (AU=(ALLEN OR FARRER))
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T S6/FULL/ALL

6/9/1 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotech Res.

0302877 DBR Accession No.: 2003-04662 PATENT

Artificial endonuclease peptide useful for detecting a nucleic acid sequence in a sample, comprises a domain which specifically binds a nucleic acid sequence and a domain which specifically binds a metal - vector-mediated recombinant protein gene transfer and expression in host cell for use in cancer therapy

9/19/02

AUTHOR: FRANKLIN S
PATENT ASSIGNEE: UNIV IOWA RES FOUND 2002
PATENT NUMBER: WO 200272807 PATENT DATE: 20020919 WPI ACCESSION NO.: 2002-723346 (200278)
PRIORITY APPLIC. NO.: US 785546 APPLIC. DATE: 20010216
NATIONAL APPLIC. NO.: WO 2002US4983 APPLIC. DATE: 20020215
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - An isolated synthetic endonuclease peptide or polypeptide (I), comprising a domain (D1) which specifically binds a nucleic acid sequence and a domain (D2) which specifically binds a hydrolytic or redox active metal, where D2 is within D1, is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid molecule (II) comprising a nucleic acid segment encoding (I), or its complement; (2) an expression cassette (III) comprising (II) which is operably linked to a promoter functional in a host cell; (3) a host cell (IV) comprising (III) or contacted with (I); (4) a vector (V) comprising (III); and (5) a composition (C) comprising (I). BIOTECHNOLOGY - Preparation: (I) is prepared by a standard recombinant method. Preferred Peptide: (I) binds Eu(III), Ce(IV), Ca(II), Mg(II), Cd(II), Cr(IV), Fe(III), Co(III), Mn(II), Cu(II) or Zn(II). (I) comprises 20 residues and a consensus EF-Hand sequence (calcium binding motif). D1 is a domain from an engrailed transcription factor. The transcription factor comprises a helix-turn-helix (HTH) domain. D1 comprises a HTH motif, relaxed HTH motif, winged HTH motif, helix-loop-strand motif or a hormone receptor motif. (I) comprises alpha-helices 2 and 3 of a HTH motif. (I) binds dsDNA, dsRNA, ssDNA, ssRNA, A-DNA, B-DNA or Z-DNA. D1 is a homeodomain. The hydrolytic metal is a transition metal. (I) binds a lanthanide, and further comprises a protein transport domain. Preferred Host: (IV) is eukaryotic or prokaryotic. ACTIVITY - Cytostatic. MECHANISM OF ACTION - Endonuclease. No biological data is given. USE - (I) is useful for cleaving a nucleic acid sequence in a sample, by contacting a sample comprising nucleic acid with (I) effective to cleave a nucleic acid sequence in the sample. (I) is also useful to detect the presence of nucleic acid in a sample, by contacting (I) with a sample comprising nucleic acid sequence suspected of containing a nucleic acid sequence recognized by (I), and determining or detecting whether (I) cleaves the nucleic acid sequence by gel electrophoresis. (I) is also useful in medical therapy (claimed). (I) is useful for treating a disease e.g. cancer. (I) is also useful as a biological probe e.g. to knock out gene function temporally e.g. for otherwise lethal mutations, and to study developmental cascades of gene function. (I) is also useful as a conformational probe, to prepare sequence-specific endonucleases e.g. restriction enzymes, for molecular biology applications, and as an agent to block or inhibit transcription for clinical antibiotic and chemotherapy applications. ADMINISTRATION - 0.01 - 100 mg/kg, preferably 0.1 - 30 mg/kg of (I) is administered through oral, systemic or parenteral e.g. rectal, buccal, vaginal and sublingual, transdermal,

subcutaneous, intravenous, intramuscular, intraperitoneal, intrathoracic, intrapulmonary and intranasal route. EXAMPLE - The 33-residue peptides P3a, P6, P7, P8 (comprising a sequence of 66 amino acids fully defined in the specification) and P9 (comprising a sequence of 74 amino acids fully defined in the specification) were based on overlays of engrailed and calmodulin crystal structures. Known protein crystal structures were oriented manually using the freeware program SwissPDBViewer (RTM) to align the fold of the homeodomain HH motifs and Ca-binding protein EF-Hand motifs. Crystal coordinates were downloaded from the Protein Data Bank (PDB) for several EF-Hand proteins, such as calmodulin (1OSA) and calcineurin (1TCO). Coordinates for the homeodomain proteins engrailed (with and without co-crystallized DNA, 2HDD and 1ENH, respectively) and antennapedia with DNA (9ANT) were obtained. The best fits (determined by inspection and RMS deviation of small helical sections) were used. P3 was a consensus EF-Hand loop, P2 was a reverse EF-Hand loop, and P4a comprised alpha2 and alpha3 of engrailed, minus the last turn(s) of alpha2 and the beta-turn, and contained calmodulin loop I. The EF-Hand and calmodulin loop I motifs had two helices at approximate right angles to one another. P4a incorporated a greater fraction of the EF-Hand turn than does P3, as well as retaining the native salt bridges (Arg-Glu) and hydrophobic contacts (Phe-Phe) between the first turns of helix E and helix F, including an aromatic Tyr group (Y(13)) in the loop, resulting in a shift in register of the Ca-binding loop to the N-terminal side. Peptides P2 and P3 was done by Dr.Suzanna Horvath of the Caltech Peptide Synthesis Facility, and P4a were synthesized by Fmoc chemistry, cleaved from the resin, and high performance liquid chromatography (HPLC) purified to greater than 95 % purity. Concentrations of stock solutions were determined by Bradford assay. Thr-Glu-Arg-Arg-Arg-Gln-Gl n-Leu-Asp-Lys-Asp-Gly-Asp-Gly-Thr-Ile-Asp-Glu-Arg-Glu-Gln-Ile-Lys-Ile-T rp-Phe (P3a) Thr-Glu-Arg-Arg-Arg-Gln-Gln-Leu-Ser-Ser-Glu-Val-Gly-Met-Th r-Cys-Ser-Gly-Cys-Ser-Gly-Gln-Ile-Lys-Ile-Trp-Phe (P6) Thr-Glu-Arg-Arg-Arg-His-Glu-Leu-Met-His-Ala-Ile-Gly-Phe-Tyr-His-Glu-Ala-Gln-Ile-Lys-Ile -Trp-Phe (P7)(101 pages)

DESCRIPTORS: recombinant endonuclease prep., isol., vector-mediated gene transfer, expression in host cell, appl. cancer therapy, DNA probe enzyme tumor hybridization DNA sequence protein sequence (22, 8)

SECTION: THERAPEUTICS-Protein Therapeutics-GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis; DISEASE-Cancer

6/9/2 (Item 2 from file: 357)

DIALOG(R)File 357:Derwent Biotech Res.

0301554 DBR Accession No.: 2003-03339 PATENT

A novel polypeptide from Drosophilia melanogaster with juvenile hormone esterase (JHE) activity, useful in identifying an arthropod control agent - vector expression in host cell for recombinant protein production useful as an insecticide and use of the vector as an insect biological control agent

AUTHOR: CAMPBELL P M; CRONE E J; SUTHERLAND T D; RUSSELL R J; OAKESHOTT J G

PATENT ASSIGNEE: COMMONWEALTH SCI and IND RES ORG 2002

PATENT NUMBER: WO 200260940 PATENT DATE: 20020808 WPI ACCESSION NO.: 2002-682680 (200273)

PRIORITY APPLIC. NO.: AU 20012821 APPLIC. DATE: 20010201

NATIONAL APPLIC. NO.: WO 2002AU101 APPLIC. DATE: 20020201

LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - A polypeptide (I) with juvenile

hormone esterase (JHE) activity and having at least 90% identity to a sequence (S1) comprising 554 amino acids fully defined in the specification or a polynucleotide encoding (I), is new. DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) identifying (M1) an arthropod control agent; (2) a crystal of (I); (3) a substantially purified polypeptide having JHE activity, comprising S1 or a sequence having at least 90% identity to S1, where the polypeptide is not more than 600 residues in length; (4) an isolated polynucleotide (II) encoding (I); (5) an antisense polynucleotide (IIa) which hybridizes under high stringency conditions to (II); (6) a double stranded RNA (dsRNA) molecule (IIb) comprising (II); (7) a fusion protein (III) comprising (I) fused to at least one other polypeptide sequence; (8) an isolated polynucleotide (IIc) encoding (III); (9) a vector (IV) comprising (II), (IIa) or (IIc), or open reading frames which upon expression forms (IIb); (10) a host cell (V) transformed or transfected with (IV); (11) a transgenic plant or transgenic non-human animal, transformed with (II), (IIa) or (IIc), or open reading frames which upon expression forms (IIb), where the plant expresses the polynucleotide or produces the dsRNA molecule; (12) an arthropod control composition (C), comprising an agent identified by M1, (I) or (IV), and an agriculturally acceptable carrier; and (13) preparing (I); and (13) a kit for identifying an arthropod control agent, comprising (I) or (II), and means for determining JHE activity. BIOTECHNOLOGY - Preparation: (I) is produced by cultivating a host cell (e.g. bacterial, arthropod or insect cell) transformed or transfected (IV) under conditions providing for expression of the polynucleotide encoding (I), and recovering the expressed polypeptide (claimed).Preferred Method: M1 comprises: (a) exposing (I) to a candidate agent, and assessing the ability of the candidate agent to modulate the JHE activity of (I); (b) exposing (I) to a binding partner which binds to (I), and a candidate agent, assessing the ability of the candidate agent to compete with the binding partner for binding to (I); (c) determining the atomic coordinates defining the three-dimensional structure of (I), selecting a candidate compound by performing rational drug design with the obtained atomic coordinates, in conjunction with computer modeling, and determining the ability of the candidate compound to modulate the JHE activity of (I); (d) exposing a polynucleotide encoding (I), to a candidate agent under conditions which allows expression of the polynucleotide, and assessing the ability of the candidate agent to modulate levels of polypeptide produced by the polynucleotide; or (e) exposing a polynucleotide having at least 90% identity to a sequence (S2) comprising 1665 nucleotides fully defined in the specification, to a candidate agent, and assessing the ability of the candidate agent to hybridize and/or cleave the polynucleotide. In M1, the binding partner (juvenile hormone) is detectably labeled. Preferred Sequence: (I) is at least 300 amino acids in length. (I) has at least 99% identity to (I). (II) comprises a sequence of 1869 or 1665 nucleotides fully defined in the specification, or a sequence which hybridizes to the above said sequence under high stringency conditions. (II) preferably has a sequence which is less than 1660 nucleotides. (IIa) comprises a catalytic domain. The dsRNA is encoded by a single open reading frame and the resulting dsRNA molecule has a stem loop structure at one end of the molecule. (III) comprises a polypeptide that enhances the stability of (I), a polypeptide that acts as an immunopotentiator to enhance an immune response to (II), or a polypeptide that assists in the purification of (III). In (IV) (which is a plasmid, capsoid or virus e.g. baculovirus), (IIa) or (IIb) is operably linked to a promoter. ACTIVITY - Arthropodicide. MECHANISM OF ACTION - Inhibitor or activator of JHE activity of (I). No supporting data given. USE - The

polypeptide, M1 or the kit are useful in identifying an arthropod control agent. (C) is useful for controlling an arthropod population (e.g. insect population), by exposing members of the arthropod population to (C). The crystal of (I) is useful in arthropod control agent design, by using the structural coordinates of the crystal to computationally evaluate a compound of its ability to modulate JHE activity of (I) (claimed). ADMINISTRATION - (C) is administered through oral, subcutaneous, intradermal, intravenous, intranasal, transdermal, intraocular or intramuscular route to animals at a dosage of 1 mug-10 mg, preferably 10 mug-1 mg/kg body weight, or applied to the environment (by spraying) for plants (dosage for plants not given). EXAMPLE - RNA was isolated from Drosophila melanogaster, and the juvenile hormone esterase (JHE) cDNA was amplified from the RNA using the oligonucleotides, JheRTPCR.F2 (5'-CGCGGATCCGCGATGCTACAACTGCTGCTTCT 3') and JheRTPCR.R2 (5'-GCTCTAGAGCTTATTACTTTTCGTTGAGTATAT 3'), that contain BamHI and XbaI sites respectively (underlined) with an additional stop codon in the case of the latter. The BamHI and XbaI digested reverse transcriptase (RT)-polymerase chain reaction (PCR) product was purified by agarose gel electrophoresis and the QIAquick PCR Purification Kit, and ligated into the pFastBac1 plasmid multiple cloning site, previously digested with BamHI and XbaI. The ligation mix was transformed into TG-1 heat shock cells and bacmid DNA was prepared and isolated. Sf9 cells were transfected with the recombinant DNA. After the initial cell wash, cells were overlayed with 1.5 ml Grace's cell culture medium without Fetal Calf Serum or antibiotics and the complete transfection mixture was added to create JHE/pFastBac1 viral supernatant (first passage) which was harvested 72 hrs after the start of transfection. The JHE/pFastBac1 viral supernatant titre was amplified in Sf9 cell mono-layer cultures grown in Grace's cell culture medium. A 25 cm2 flask was seeded to high confluence (approximately 2x106 cells) and 2 ml of JHE/pFastBac1 viral supernatant (first passage) was added to a total of 3 ml of Grace's cell culture medium and incubated at 27degreesC for 72 hrs. The culture was harvested and centrifuged. The presence of JHE products in the supernatant was then determined. (67 pages)

DESCRIPTORS: Drosophila melanogaster recombinant juvenile-hormone-esterase protein prep., 3D structure coordinate, capsoid, baculo virus vector-mediated gene transfer expression in bacterium, Sf9 cell, sense, antisense oligonucleotide, fusion protein, transgenic plant, non-human transgenic animal, computer modeling, promoter, reverse transcription polymerase chain reaction, appl. biological control agent, insecticide enzyme DNA amplification insect cell culture Spodoptera frugiperda arthropod animal DNA sequence protein sequence pesticide (22, 07)

SECTION: AGRICULTURAL BIOTECHNOLOGY-Biological Control Agents-GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis; AGRICULTURAL BIOTECHNOLOGY-Plant Genetic Engineering-BIOMANUFACTURING and BIOCATALYSIS-Animal/Plant Cell Culture; GENETIC TECHNIQUES and APPLICATIONS-Transgenic Animals and Animal Models-AGRICULTURAL BIOTECHNOLOGY-Pesticides

6/9/3 (Item 3 from file: 357)

DIALOG(R)File 357:Derwent Biotech Res.

0299084 DBR Accession No.: 2003-00868 PATENT

Novel array of clones, comprising multiple group of vessels each containing a clone which contains exogenous gene segment within gene or allele of gene of genome such that all genes or alleles of the gene is disrupted - vector-mediated reporter gene transfer and expression in host cell

for gene expression analysis and functional genomics

AUTHOR: LOFQUIST A; FINNEY R E; LEUNG D
PATENT ASSIGNEE: LOFQUIST A 2002
PATENT NUMBER: WO 200253732 PATENT DATE: 20020711 WPI ACCESSION NO.: 2002-583614 (200262)
PRIORITY APPLIC. NO.: US 258388 APPLIC. DATE: 20001228
NATIONAL APPLIC. NO.: WO 2001US50515 APPLIC. DATE: 20011228
LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - An array of clones (I), comprising multiple groups of vessels, of which at least two of the vessels each contain a clone, where each clone has an exogenous segment within a gene or allele of a gene of its genome, so that all genes or alleles of the gene is disrupted, and is arranged in the array in predetermined fashion, is new. DETAILED DESCRIPTION - An array of clones (I), comprising multiple groups of vessels, of which at least two of the vessels each contain a clone, where each clone contains an exogenous segment within a gene or allele of a gene of its genome, so that all genes or alleles of the gene is disrupted, and is arranged in the array in predetermined fashion. Optionally, (I) comprises multiple groups of vessels, of which at least two of the vessels each contain a clone, where each clone contains an first exogenous segment within a first gene of its genome, so that the first gene is disrupted, and a second exogenous segment within a second gene of its genome, so that the second gene is disrupted. INDEPENDENT CLAIMS are also included for the following: (1) a construct (II) comprising functional elements that, when transcribed, produce a eukaryote mRNA transcript that contains a component such as an origin of replication and a host cell selection marker; (2) a library of constructs comprising more than 10 of the constructs, where each construct of the library produces, upon transcription, an mRNA transcript containing an exogenous origin of replication or a host cell selection marker, and where at least some of the mRNA transcripts produced by the constructs represent different gene sequences, or each of the mRNA transcripts produced by the constructs represent a region of a cell genome that does not encode a polypeptide; (3) a homologous recombination vector comprising: (a) a splice acceptor sequence or an internal ribosome entry site (IRES) sequence; (b) an origin of replication or a host cell selection marker; (c) a unit for facilitating termination and polyadenylation of an endogenous polynucleotide; (d) a first genomic fragment recovered from a cell; and (e) a second genomic fragment recovered from a cell, where (b) is downstream of (a) and upstream of (c), and where the first genomic fragment is upstream of (a) and the second genomic fragment is downstream of (c); (4) a linear construct comprising in the 5' to 3' order: (a) a first nucleotide sequence that is homologous to a first genomic sequence of a cell; (b) a construct comprising an origin of replication that is exogenous to the cell; and (c) a second nucleotide sequence that is homologous to a second genomic sequence of the cell; (5) a positive switch homologous recombination vector; (6) a cell (a single allele-disrupted cell) (III) comprising an allele of a first gene into which an exogenous polynucleotide has been integrated, where the exogenous polynucleotide contains an origin of replication or a selectable marker upstream of an unit for facilitating termination and polyadenylation of an endogenous polynucleotide and downstream from a transcription initiation sequence; (7) a cell (two-allele disrupted cell) (IV) comprising: (a) a first allele of a first gene into which an exogenous polynucleotide has been integrated by a method other than homologous recombination; and (b) a second allele of the first gene into which a homologous recombination event has occurred; (8) a multiple-gene disrupted cell (V), comprising: (a) all alleles of a first gene contain an integrated construct or its portion; and (b) all

alleles of a second gene contain an integrated construct or its portion, where the construct comprises an exogenous origin of replication or a host cell selection marker; (9) a library of (III), comprising at least two (III); (10) a library of (IV) comprising at least two (IV); (11) a library of (V) comprising at least two (V); (12) a collection of at least two cells, each comprising an allele of a first gene into which an exogenous polynucleotide has been integrated, where the exogenous polynucleotide contains an origin of replication or a selectable marker that is: (a) upstream of an unit for facilitating termination and polyadenylation of an endogenous polynucleotide; and (b) downstream from a transcription initiation sequence, where each cell comprises a different gene into which an exogenous polynucleotide is integrated; (13) determining the function of a gene, comprising: (a) providing a cell containing at least two disrupted alleles of a gene; and (b) comparing biological traits to those of a second cell in which no alleles of the gene are disrupted, where an allele of the first cell contains an exogenous polynucleotide integrated by a method other than homologous recombination and at least one other allele contains an exogenous segment integrated by homologous recombination, or each allele of the first cell is disrupted by a homologous recombination vector; (14) selecting a compound that regulates the expression of a reporter marker integrated into at least one allele of a gene in a cell, comprising: (a) contacting a compound with at least one cell of a cell library; and (b) comparing fluorescent light intensity of a reporter marker sequence integrated into the cell before and after contacting the compound, where the cell comprises an origin of replication or a host cell selection marker integrated into its genome; (15) determining effectiveness of a double-stranded RNA molecule comprising: (a) introducing a construct that comprises a promoter, a polynucleotide of interest, an IRES sequence, and a reporter marker into a cell that has one allele disrupted by an exogenous polynucleotide; (b) determining the activity or expression level of the reporter marker; (c) introducing into the cell a double-stranded RNA molecule designed to a portion of the polynucleotide of interest; and (d) determining the activity or presence of the reporter marker; and (16) integrating a trap construct into a cell genome, involves introducing into a cell: (a) a trap construct; and (b) a transposase enzyme that recognizes inverted repeat sequences in the trap construct, where the transposase induces the integration of a part of the construct into the genome. WIDER DISCLOSURE - (1) an array of cells comprising multiple groups of the cells of which at least two of the vessels each contain cells containing a gene whose expression is inhibited or altered at transcriptional, translational or protein level, and is arranged in predetermined fashion in the array; (2) a vector library comprising at least two vectors, each vector comprising a first nucleotide sequence homologous to a first genomic sequence of a cell, (a construct comprising a marker sequence, and a second nucleotide sequence homologous to a second genomic sequence of the cell; (3) making a cell in which at least two alleles of a gene, are disrupted by a construct exposed to the cell; (4) selecting a drug candidate that regulates expression of a marker integrated into at least one allele of a gene in a cell; (5) interfering with the operation of a target gene in a cell; (6) a library of double stranded RNA molecules, where each dsRNA in the library is associated with the target gene or target genes; (7) modulating the expression of target gene by introducing double stranded RNA molecule that shares sequence homology with the actual and/or predicted mRNA transcript of a target gene; and (8) up regulating expression of target genes by introducing into a cell double stranded RNA to modulate expression of a gene. BIOTECHNOLOGY - Preferred Construct: (II) comprises a splice acceptor site, and a

cassette sequence such as: (a) a transcriptional termination sequence; and (b) a splice donor site; It also comprises a cell selection marker sequence, and an origin of replication, where the origin of replication and the cell selection marker sequence located downstream to the 5'-end of the splice acceptor site and upstream to the 3'-end of the cassette sequence, and where the origin of replication is exogenous to the splice acceptor site or the cassette sequence. More preferably, the construct comprises a transcriptional initiation sequence, a splice donor site, a cell selection marker sequence. and an origin of replication. The origin of replication and the marker sequence are located downstream to the 5' end of the transcriptional initiation sequence and upstream to the 3' end of the splice donor site, and where the origin of replication is exogenous to the transcriptional initiation sequence or the splice donor site. (II), homologous recombination vector, positive switch homologous recombination vector or linear construct, further comprises at least one inverted repeat sequence that is targeted by a transposase enzyme. Preferred Vector: The vector of (5) comprises: (a) a splice acceptor sequence or an internal ribosome entry site (IRES) sequence; (b) a first termination sequence; (c) a positive selective marker; (d) a first genomic fragment recovered from a cell; and (e) a second genomic fragment recovered from a cell, where the elements are arranged so that (b) is upstream of (a), (c) and (d) and (e) is downstream of (a), (c) and (d). USE - (III) is useful for recovering at least a portion of a gene allele of a cell genome, comprising providing (III) in which at least one allele of a gene comprises a construct having an origin of replication or a host cell selection marker, recovering a nucleic acid molecule containing the construct, and isolating the nucleic acid molecule, where the nucleic acid is derived from the allele flanks either the 5'-end, the 3'-end or both ends of the construct. (IV) is useful for determining the function of a gene, by providing (IV) containing at least two disrupted alleles of a gene, and comparing biological traits of the cell to those of a second cell in which no alleles of the gene are disrupted. (All claimed).(115 pages)

DESCRIPTORS: clone array construction, homologous recombination, vector-mediated reporter gene transfer, expression in host cell, splice acceptor sequence, internal ribosome entry site, DNA library, gene expression analysis, specific gene allele det., appl. functional genomics genomics DNA sequence (22, 02)

SECTION: GENETIC TECHNIQUES and APPLICATIONS-Genomic Technologies-GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis

6/9/4 (Item 4 from file: 357)

DIALOG(R)File 357:Derwent Biotech Res.

0297045 DBR Accession No.: 2002-18892 PATENT

Novel isolated membrane fusion protein isolated from reoviruses and the p14 protein from reptilian reovirus isolated from python or p16 protein from aquareovirus isolated from salmon, useful for promoting membrane fusion - with application in membrane fusion in e.g. hybridoma construction or in transfection by means of lipofection e.g. for gene therapy

AUTHOR: DUNCAN R

PATENT ASSIGNEE: FUSOGENIX INC 2002

PATENT NUMBER: WO 200244206 PATENT DATE: 20020606 WPI ACCESSION NO.: 2002-527699 (200256)

PRIORITY APPLIC. NO.: CA 2325088 APPLIC. DATE: 20001201

NATIONAL APPLIC. NO.: WO 2001CA1702 APPLIC. DATE: 20011130

LANGUAGE: English

ABSTRACT: DERWENT ABSTRACT: NOVELTY - An isolated membrane fusion protein (I) isolated from reoviruses isolated from poikilothermic hosts, the p14 protein from reptilian reovirus (RRV) isolated from python, or p16 protein from aquareovirus (AQV) isolated from salmon, encoded by the genome of the family Reoviridae, is new. DETAILED DESCRIPTION - An isolated membrane fusion protein (I) encoded by the genome of family Reoviridae, and comprising at least one transmembrane domain. The amino acid sequence of (I) is free of fusion peptide motif I of 17-28 residues long (the motif has a hydrophobicity value from 0.6 to 0.7, and 29%-43% of alanine plus glycine content). The amino acid sequence of (I) is also free of fusion peptide motif II of 16-28 residues long, where the motif has a hydrophobicity value from 0.3 to 0.4, 29%-43% of alanine plus glycine content, and a heptad repeat. INDEPENDENT CLAIMS are also included for the following: (1) an isolated polynucleotide (II) encoding (I), or comprising a sequence (S1) of 1501 or 1398 nucleotides fully defined in the specification, degenerate variants of S1 encoding the same amino acid sequence or splice variant of S1; (2) a cell (III) containing (I) or (II); (3) a liposome (IV) containing (I) or (II); and (4) an antibody (Ab) against (I). WIDER DISCLOSURE - Also disclosed are: (1) an expression cassette containing (II) under the control of elements required for expression; (2) an expression vector containing the above said expression cassette; (3) producing (I) by culturing a host cell transformed or transfected with the above said expression cassette and/or vector under conditions that allow expression of the DNA molecule, and recovering the encoded polypeptide; and (4) compositions comprising (I). BIOTECHNOLOGY - Preferred Polypeptide: (I) Further comprises an amino acid sequence having at least 33% identity overall to (I), and a transmembrane domain whose amino acid sequence has at least 60% identity to the transmembrane domain of (I). (I) is encoded by the genome of a reovirus selected from orthoreovirus and aquareovirus. The reovirus naturally infects a poikilothermic host. (I) has an isoelectric point at least7. (I) further comprises a positive cluster comprising at least three positively charged amino acid residues within a contiguous sequence of at most 25 residues, where the contiguous sequence is within at most 100 residues flanking the transmembrane domain at the C-terminal side. (I) further comprises a non-transmembrane domain which comprises a polyproline motif comprising at least 3 contiguous proline residues, and a fatty acylation sequence. The amino acid sequence of the transmembrane domain has at least 60% identity to amino acids 39-57 of a sequence (S2) comprising 125 amino acids fully defined in the specification. (I) further comprises a sequence having at least 33% identity to a sequence (S3) comprising 158 amino acids fully defined in the specification. the transmembrane domain has at least 60% identity to the sequence 5' WAIPPLAICCCCCICCTGGLYLV 3' or YIALASVILVTLVISLIWNCLGTGLIL 3' of S3. The fatty acylation sequence is the myristylation consensus sequence (initiator Met removed) Gly1-(AA)2-8, where (AA)2-8 are small uncharged residues, and AA6 is not P. Preferably, AA5 is S or T. Preferred Sequence: (II) is operatively associated with an inducible promoter. USE - (I) Is useful for promoting fusion between two or more membranes, by contacting the membranes to be fused e.g. cell membranes, liposome membranes and proteoliposome membranes, with (I). Immortalized cells are fused with primary B cell or T cell, for producing hybridoma cell which produces monoclonal antibodies, cytokines and immune modulators (claimed). Fusion of membranes is useful in clinical, industrial and basic research situations. EXAMPLE - The viral genomic dsRNA segments were isolated from concentrated virus stocks pretreated with RNase and DNase to remove extra-virion contaminating cellular nucleic acids. Virus

particles were disrupted using 1% sodium dodecyl sulfate (SDS) and the viral dsRNA was isolated. Aliquots of genomic dsRNA (20 mug) were poly-A-tailed using Escherichia coli poly-A polymerase, the tailed RNA was fractionated by agarose gel electrophoresis, and individual genomic segments were isolated. The tailed S class genome segments were used as templates for reverse transcription, using Superscript reverse transcriptase and an oligo-dT primer. Aliquots of the plus and minus strand cDNAs were used as templates for polymerase chain reaction (PCR) amplification using Vent polymerase and an oligo-dT primer containing a NotI restriction enzyme site. The products of the PCR reaction were digested with NotI, size-fractionated on agarose gels, and products corresponding to the full length S genome segments were gel-purified. The individual, NotI-digested, double-stranded cDNAs were cloned into the NotI site of pBluescript and used as templates for sequencing. The cloned cDNAs were sequenced using an automated DNA sequencer. The reptilian reovirus (RRV) and aquareovirus (AQV) cDNA clones were subcloned into the eukaryotic expression vector pcDNA3 under the control of the CMV promoter. Plasmid DNA was isolated and purified. Plasmid DNA (1 microg) was mixed with Lipofectamine (RTM) (3 microl) and used to transfect sub-confluent cell monolayers grown in 12 well cluster plates. Transfected cell monolayers were incubated at 37degreesC for 24-48 hour before being fixed with methanol and stained using a water-soluble Wright-Giemsa stain. Cell fusion was assessed by light microscopy of stained monolayers and syncytial foci were photographed at 100 x magnification. Using this protocol, the S1 genome segment of RRV and genome segment 7 of AQV were determined to encode the fusion proteins of these viruses. The unrelated fusion proteins responsible for the cell-cell fusion induced by RRV and AQV have been identified. These proteins were referred to as p14 (for RRV) and p16 (for AQV) to reflect their approximate predicted molecular weights. The genes encoding p14 and p16 were cloned and sequenced. The sequence-predicted structural organization of these proteins were analyzed, and the membrane fusion properties were directly demonstrated. The cDNA sequences comprised 1501, or 1398 nucleotides fully defined in the specification, and the predicted translation products comprised a sequence of 125, 349 or 158 amino acids fully defined in the specification.(82 pages)

DESCRIPTORS: reo virus membrane fusion protein prep., python reptile reo virus p14 protein, salmon aquareo virus p16 protein prep., vector-mediated gene transfer, expression in host cell, liposome, appl. hybridoma construction, monoclonal antibody prep., cytokine prep., immunomodulator prep., lipofection, pot. gene therapy animal fish transfection DNA sequence protein sequence (21, 51)

SECTION: GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis-BIOMANUFACTURING and BIOCATALYSIS-Animal/Plant Cell Culture; PHARMACEUTICALS-Antibodies-THERAPEUTICS-Gene Therapy; THERAPEUTICS-Protein Therapeutics

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Producing herpes simplex virus amplicon particles with transgene encoding therapeutic product by cotransfecting host cell with vectors essentially having transgene and sequence encoding virion host shutoff protein - vector-mediated ribozyme or antisense RNA transfer and expression in host cell for gene therapy

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LANGUAGE: English
ABSTRACT: DERWENT ABSTRACT: NOVELTY - A host cell cotransfected with amplicon vector, comprising a herpes simplex virus (HSV) origin of replication, HSV cleavage/packaging signal (C/PS), and heterologous transgene, vectors individually or collectively encoding all essential HSV genes but excluding all C/PS, a virion host shutoff (vhs) protein expression vector, and herpes simplex virus (HSV) amplicon particles (II), is new. DETAILED DESCRIPTION - Producing (M1) (II), by cotransfecting host cell with an amplicon vector (Ia) having HSV origin of replication, HSV C/PS, and heterologous transgene (TG) expressible in a patient, one or more vectors (Ib) individually or collectively encoding all essential HSV genes but excluding all C/PS, and a vhs expression vector (Ic) encoding virion host shutoff protein; and isolating (II). INDEPENDENT CLAIMS are also included for the following: (1) (II) produced by (M1); (2) a system (III) for (M1) comprising (Ia), (Ib) and (Ic); and (3) a kit (IV) for (M1) comprising (Ia), (Ib), (Ic), a population of host cells susceptible to transfection by (Ia), (Ib) and (Ic), and directions for transfecting the host cells under conditions to produce (II). BIOTECHNOLOGY - Preferred Method: In (M1), the isolated (II) are substantially pure; (Ic) preferably comprises a DNA molecule encoding vhs protein, a promoter element operatively coupled to the 5'end of the DNA molecule; and a transcription termination element operatively coupled to the 3' end of the DNA molecule, where the vhs protein is from virion shutoff protein of HSV-1, HSV-2 and HSV-3, bovine herpes 1 and 1.1, gallid herpesvirus 1 and 2, suid herpesvirus 1, baboon herpesvirus 2, pseudorabies virus, cercopithecine herpesvirus 7, melegrid herpesvirus 1, equine herpesvirus 1 and 4, more preferably vhs protein of HSV-1, HSV-2 or HSV-3, where (Ic) preferably comprises a DNA molecule encoding the HSV vhs protein operatively coupled to its native transcriptional control elements. The host cell expresses a VP16 protein which is from VP16 of HSV-1, HSV-2, bovine herpesvirus 1 and 1.1, gallid herpesvirus 1 and 2, meleagrid herpesvirus 1 or equine herpesvirus 4. TG encodes a therapeutic TG product which is preferably a protein selected from receptors, signaling molecules, transcription factors, growth factors, apoptosis inhibitors, apoptosis promoters, DNA replication factors, enzymes, structural proteins, neural proteins and histone or non-histone proteins; or an RNA molecule selected from antisense RNA, RNAi, and an RNA ribozyme. The isolated (II) are preferably present at a concentration of greater than 1x106 particles/ml. (M1) further comprises concentrating the isolated (II) to a concentration of at least 1x107 particles/ml. Preferably the host cell is transfected with a vector encoding VP16 protein, prior to co-transfecting, where the transfection is carried out at least 4 hours and the host cell is made to stably express VP16 protein. Preferred System: (III) further comprises a host cell which stably expresses a VP16 protein, or a vector encoding VP16 protein. Preferred Kit: (IV) further comprises a host cell which stably expresses a VP16 protein, or a vector encoding VP16 protein. ACTIVITY - Nootropic; Neuroprotective; Antiparkinsonian; Cerebroprotective; Antitumor; Anticonvulsant. No biological data provided. MECHANISM OF ACTION - Gene therapy. No supporting data is given. USE - (II) is useful for treating a neurological disease or disorder such as lysosomal storage disease, Lesch-Nyhan syndrome, amyloid polyneuropathy, Alzheimer's disease, retinoblastoma, Duchenne's muscular dystrophy, Parkinson's disease, Diffuse Lewy body disease,

stroke, brain tumor, epilepsy and arteriovascular malformation, comprising exposing neural or pre-neural cells of a patient preferably a human to (II) preferably present in a carrier; for inhibiting development of a neurological disease or disorder, comprising exposing neural cells of a patient susceptible to development of a neurological disease or disorder to (II); or expressing therapeutic gene product in a patient comprising exposing patient cells to (II), where the therapeutic TG product is expressed in vivo in the cells, and the exposing is carried out in ex vivo using pre-neural cells or in vivo by administering (II) directly to neural cells preferably under conditions effective for infective transformation of the cells. The method further comprises introducing transformed the cells into the patient (all claimed). ADMINISTRATION - (II) administered through intraparenchymal, intramuscular, intravenous, intracerebroventricular, subcutaneous or intramucosal route (claimed). No dosage detail is given. ADVANTAGE - Co-transfection of vhs expression vector with the amplicon and packaging reagents results in a 10-fold higher amplicon titer and do not exhibit the pseudotransduction phenomenon. The HSV transcriptional activator VP16 was introduced into packaging cell prior to the packaging components, to further enhance packaging efficiency. Pre-loading of packaging cells with VP16 led to an additional enhancement of amplicon titers, an effect that did not occur in the absence of vhs. Increased helper virus-free amplicon titers resulting from these modifications makes the in vivo transduction experiments more feasible. EXAMPLE - No relevant example is given. (94 pages)

DESCRIPTORS: herpes simplex virus vector prep., vector-mediated virion host shutoff protein, VP16 protein, receptor, signaling molecule, transcription factor, somatotropin, apoptosis inhibitor, apoptosis promoter, DNA replication factor,enzyme, structural protein, neural protein, histone, non-histone protein, ribozyme, antisense RNA gene transfer, expression in host cell, packaging cell culture, appl. neurological disease, lysosomal storage disease, Lesch-Nyhan syndrome, amyloidpolyneuropathy, Alzheimer disease, retinoblastoma, Duchenne muscular dystrophy, Parkinson disease, Diffuse Lewy body disease, stroke, brain cancer, epilepsy, arteriovascular malformation gene therapy herpes virus hormone RNA enzyme tumor (21, 26)

SECTION: THERAPEUTICS-Gene Therapy-GENETIC TECHNIQUES and APPLICATIONS-Gene Expression Techniques and Analysis; DISEASE-Cancer-BIOMANUFACTURING and BIOCATALYSIS-Animal/Plant Cell Culture; DISEASE-Cardiovascular-DISEASE-Central Nervous System; DISEASE-Neuromuscular System-DISEASE-Endocrine/Metabolic System; DISEASE-Other Diseases

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